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CLINICAL AND MORPHOLOGICAL EVOLUTION OF THE INDUCED EXPERIMENTAL ARTHRITIS IN *Rattus novergicus albinus*

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ABSTRACT

The models of experimental arthritis become important in the inquiry of different therapeutical alternatives and briefing of articulate pathogenesis. The possibility of measuring the injury of the articular cartilage makes the experimental model relevantly important, as well as the systemic biological effects that involve the different therapeutics: The radiological and histological aspects of the cartilage were researched in the model of Zynoman-induced arthritis in Rattus novergicus. Rats were submitted to the intra-articular injection (1.0ml) and sacrificed at different times, under anesthesia. The knee joints were surgically removed and processed for coloring in hematoxylin eosin (H&E). The radiographic analyses were carried out through images obtained with dental periapical film. The animals presented serious and gradual synovitis associated to the injury of the cartilage that was evaluated up to 14 days after the stimulation injection. The arthritis model by Zymosan allows the study of the inflammatory alteration of the synovial tissue and of the cartilage. In the presence of Zymosan, the juxtarticular and periarticular tissues develop similar alterations to those found in the autoimmune diseases.

Key word: Rheumatoid arthritis RA; induced arthritis by Zymosan AZy; anti-tumor necrosis factor (TNF)-α.

INTRODUCTION

The rheumatoid arthritis (RA) is a chronic systemic disease, characterized by inflammatory incidents in the sinovyal membranes and articular structures. Fibrinoid degeneration of collagen fibers in mesenchymas tissues, atrophy and rarefaction of osseous structures [19] take place. Autoimmune mechanisms have been listed as indicative of this pathology that affects the joints of the hands, fists, elbows, shoulders, feet, ankles and knees, and can acquire a systemic character, developing extra-articular, cardiac, pulmonary, vascular and ophthalmologic manifestations [30]. It is believed that 1% of the population in Brazil is affected by this pathology, congregating an increasing universe of carriers of some articular deformity, generating incapacities at a predominantly professionally active age (between 30 and 50 years old) [6]. Generally, rheumatoid arthritis appears after the age of 30, occurring three times more in women than in men [1]. Despite the high occurrence, the RA etiology remains unknown and it is believed to be related to genetic factors [8, 18] that determine the individuals whose immunological system is susceptible to being attacked by the disease. Genes related with RA are not simply or directly inherited. Infectious, chemical or mechanical hormones or environmental factors can contribute to the development of the pathology in susceptible, genetically determined people [7, 20, 23-25]. The disarrangement of the immune system that occurs in RA is extremely complex. Autoantigens cause

activation, initiating an inflammatory process that stimulates the release of cytosine. Most of the cells can produce cytosine, but, the macrophages play a major role because the cytocines derived from the macrophage alter the behavior between the endothelial cells and leukocytes (TNFα), recruitment of leukocytes (IL-8), reply in the acute phase (IL-6, IL-1) and immunological function (IL-1, il-6, IL12). Interlucina-1(IL1) mediates the inflammatory reply in the natural immunity and stimulates the osseous absorption, increasing the precursory number of cells of osteoclasts, besides stimulating the production of prostaglandins and collagens by the fibroblasts and osteoblasts. Interlukine-6 (IL-6). stimulates the proliferation of T cells, the activation of the natural mechanism of cellular death and cytotoxicity.

The anti-tumor necrosis factor (TNF)-α also known as cachectin, is mainly produced by macrophages, but can also be set free for lymphocytes and mastocytes. Besides inducing production of collagens and prostaglandins, it is chemotactic for inflammatory cells [28]. The TNFα regulates the synthesis of other cytokines such as the IL-6 and the IL-1. Pro-inflammatory cytokines such as the factor of tumor necrosis (TNF) and the interleukin I (IL-1) induce hyperactivity of auxiliary T cells. The result includes expansion of the cellular immunity and synthesis of auto-antibodies by lymphocytes B [27]. Occurrences such as activation of the complement (generated by auto-antibodies that may be deposited in the tissue), aggregated platelets and synthesis of prostaglandins culminate with intense vasculitis of small veins in the RA evolution process in tissues. The synovial alterations in RA are edema and accumulation of lymphocytes, plasmacytes and macrophages; concomitantly with increase of the vascularity and exudates in the articular space, resulting in fibrin nodules that float in the joint (rice corpuscle) [4,10]. The synovial covering cells, composed of three layers, suffer hyperplasia and form layers with 8 to 10 cells thick that results in covering synovial tissues with numerous villosities. As the synovium suffers hyperplasia and hypertrophy, it is elongated on the articular cartilage and as from this incident known as pannus [23, 24]. Pannus produces erosion of the articular cartilage and of the underlying bone through the action of collagens produced by it that penetrates into the subchondral bone affecting tendons, ligaments and the articular capsule. When the articular cartilage is destroyed, it suffers fibrosis and develops into ankylosis [9]. Treatment of the aggressive RA cases is onerous for public funds, a complication that makes the disease particularly worrying. The immediate beginning of the treatment and the precocious diagnosis are basic for the control of the activity of the disease and to prevent functional incapacity and irreversible articular injury [6]. In order to understand the processes that involve the evolution of the autoimmune disease, so that they can be prevented or interrupted, a search was made for experimental models, to reproduce the majority of the findings of RA and to minutely research the evolution of the disease as well as the effects on the tissue of an autoimmune aggression. In order for the animal models to be accepted, they must supply information on the hystopathological, biomolecular and genetic aspects of autoimmunity. They must point out characteristics with clinical, pathological and radiological findings, similar to those of RA and with few systemic manifestations. The beginning, the gravity and incidence of the disease must be trustworthy, with reproduction of the arthritis within a short period to allow for fast experimental protocols that are really measurable [2]. Experimental arthritis has been induced through substances, such as adjuvant, fragments of streptococcal cellular wall, type II collagen, retrovirus, lactobacillus, micoplasma, mycobacteria [16] and Zymosan (Zy) [10,13]. Zymosan is a polysaccharide derived from the wall of the Saccharomyces cerevisiae fungus that possesses in its structure an organosulphorous composition with immunomodulatory properties, the β- glucan, widely used in vivo experiments to study the behavior of leukocycitatic cells in inflammatory process [10]. Through the dectin1 receiver, Zymosan is recognized for macrophages and interacts with the TLR2 receiver [27] that has an important role in the development of acquired immune answers [12].

MATERIAL AND METHODS

This study was approved by the CEEA-IB/UNICAMP in 04/05/2005 under no. 811-1. In the study of arthritis in Wistar rats (*Rattus norvegicus albinus*), 80-day old females with 250g each from the central vivarium of the Medical Science College of the Campinas State University were divided into group, control and treated. The group control was submitted to intra-articular injection of saline solution and the induced one (AZy) received intra-articular Zymosan in the right knee. The inductive vehicle used was Zymosan - Saccharomyces cerevisiae (Sigma, St Louis, MO, USA) and chloral Hydrate anesthesia at 10 %, (400mg/kg intraperitoneal). The AZy groups received the Zymo-

san injection (1mg) under anesthesia, in 50µl of saline solution at 0.9% in the infra patellar region of the right knee and group C, 50µl of saline solution at 0.9%. The experiments were concluded at different times, 3, 7, 14 days of arthritis induction, followed by sacrifice by deep anesthetic. Inflammatory signs were collected through articular cirtometry (edema), articular heat, redness and loss of function (time of nociception-paw rise) for inclusion of the animal in the experiment. The parts containing the right femur-pattelar joint were collected, identified, immersed in formalin solution at 10% for 48 hours, decalcified by EDTA and after 72 hours, they were included in paraffin. The histological 5-micrometer cuts obtained were stained by the hematoxylin-eosin method and analyzed in Carl Zeiss optic photomicroscope in the Anatomy department. Radiological images were collected using dental periapical radiographic films. The Accurate Fischer test was used for the statistical analysis of the results and the 5% significance level was adopted. The surgical procedures and treatments were carried out according to The Guide for the Care and Use of Laboratory Animals (DHEW Publication, Bethes MD, U.S.A., 1980).

RESULTS

Inflammatory signals

Heat

The average values of articular temperature collected on the 3rd day of arthritis induction by Zymosan (AZy), presented, in relation to the control, an increase in 24hrs after the induction of AZy, but which remained unchanged until the 3rd day when they gradually diminished until the 14th day (Table 1).

Table 1
Average temperature values of the groups given in centigrades.

	Control	24 hours	3 days	7 days	14 days
N=10	36.840	39.270	39.250	38.070	37.540

Loss of function

After the end of the anesthesia sedation, the animals were stimulated to walk and the animals induced with intra-articular Zy, kept their paws raised for 8 hours. (8 TEP = h - time of paw rise).

Edema

The presence of articular tumor was observed through cirtometric values of the articular edema (Table 2).

Table 2 - Cirtometric Values in the groups
The cirtometric averages of the increase in value as from the 3rd day of arthritis induction.

N=10 Nº of animals per group	control	3 days zy	7 days zy	14 days zy
Average	5.88	6.61	7.18	7.16

Total no. = 40
Significant statistic index on the Fisher scale P-value = 0.4552

The average cirtometric values of the 14th day of AZy, increased by 52.7% in relation to the average values of the control group.

Pain

The articular pain perceived to palpation was present in the first 24 hours, remaining unchanged until the 14th day, statistically acquiring insignificant values with p=0.4552 on the Fischer scale.

Redness

Together with the edema and temperature increase, the joint submitted to intra-articular Zymosan injections, takes on moderate indexes in 24 hours to the 3rd day, going onto intense on the 7th day and again moderate on the 14th day of inflammation. P-value=0.0523 on the Fisher scale.

Radiological analysis

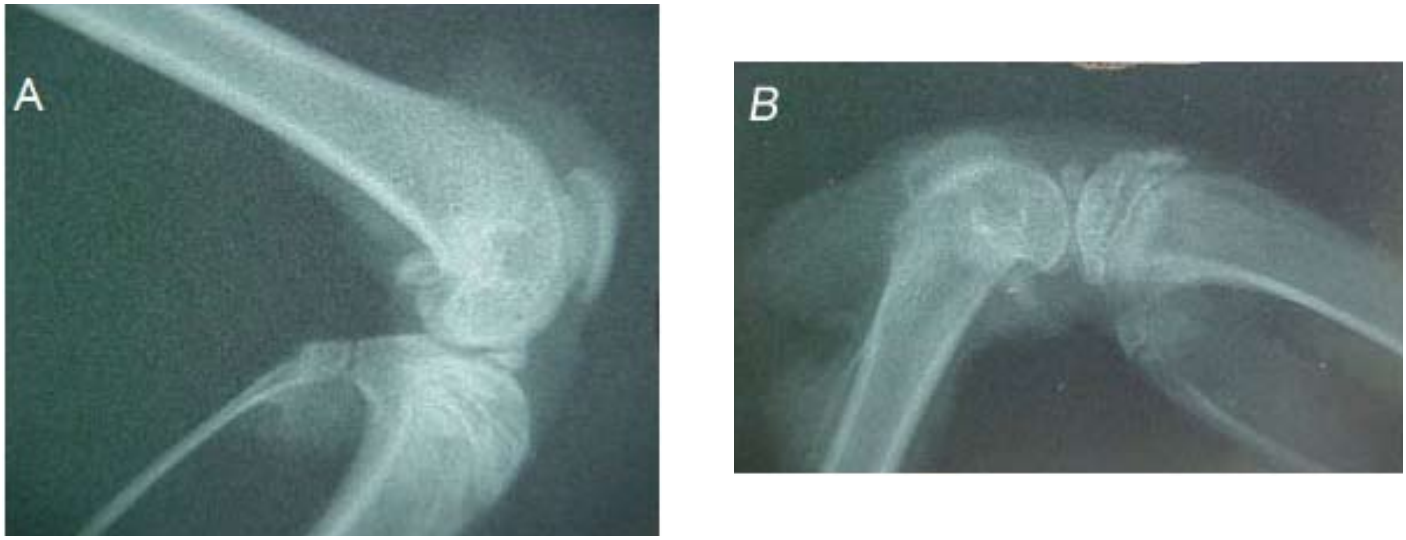

Figure. 1 Comparation of roentgenogaph of normal rat knee (A) showing intra-articular line preserved, showing that the distance between the osseous extremities seen in the x-ray is kept by the thickness of the articular cartilage present and intact, and with anatomically positioned patella score bar : 1cm. (B) group experimentally induced by Azy, one can observe subchcondral translucent areas with erosion in the above located patella, adhered to the femoral condyle, diminishing the group experimentally induced by Azy, one can observe subchcondral translucent areas with erosion in the above located patella, adhered to the femoral condyle. bar: 1cm.

In the radiological analysis, the control group presents normal radiological aspects with intra-articular line preserved, showing that the distance between the osseous extremities seen in the x-ray is kept by the thickness of the articular cartilage present and intact, and with anatomically positioned patella (fig.1 A). In the group experimentally induced by Azy, one can observe subchcondral translucent areas with erosion in the above located patella, adhered to the femoral condyle, diminishing the space between the femur and the supra patellar tendon, showing compression of soft parts. (fig. 1 B)

Histological analysis

It was observed that in the microscopic analysis of the collected material on the 3rd day after the inoculation with Zymosan there was a presence of moderate inflammation with synovial and villous hyperplasia in the juxtaposed soft tissues, producing a chronic, unspecified, inflammatory process (fig.2.B,C). On the 7th day of AZy, an intra-articular region is noticed with infiltrated, unspecific, diffuse inflammatory lymphoplasmocytes with beginning of inflammatory flake organization. The great difference between the 3rd and 7th day is that there is osseous reactionary proliferation. In the 14-day induced arthritis, aggregate-forming infiltrated, inflammatory focal lymphoplasmocytes are present. The inflammation is intense, located in the synovial, muscular, periarticular tissue. The inflammation focus is organized with predominance of giant cells and marginal erosions in the osseous tissue appear. There is presence of pannus and the articular surface is destroyed with reactive neoformed osseous tissue (fig. 2 D,E).

DISCUSSION

Animal models of arthritis have been used so that the elements that participate in the arthritic process and its mechanisms of action in the biological structures of the patients are understood [16]. The development of new drugs [5] or physical kinds of treatment find powerful tools to study pathological changes in biological tissues, systems and organs in the animal models [16,17-30]. Although the articular inflammation points out its presence with more destruction, the reversal of the inflammatory and erosive process can also be seen through the different mediators involved in these processes [3,7,8-12]. When choosing a model of experi-

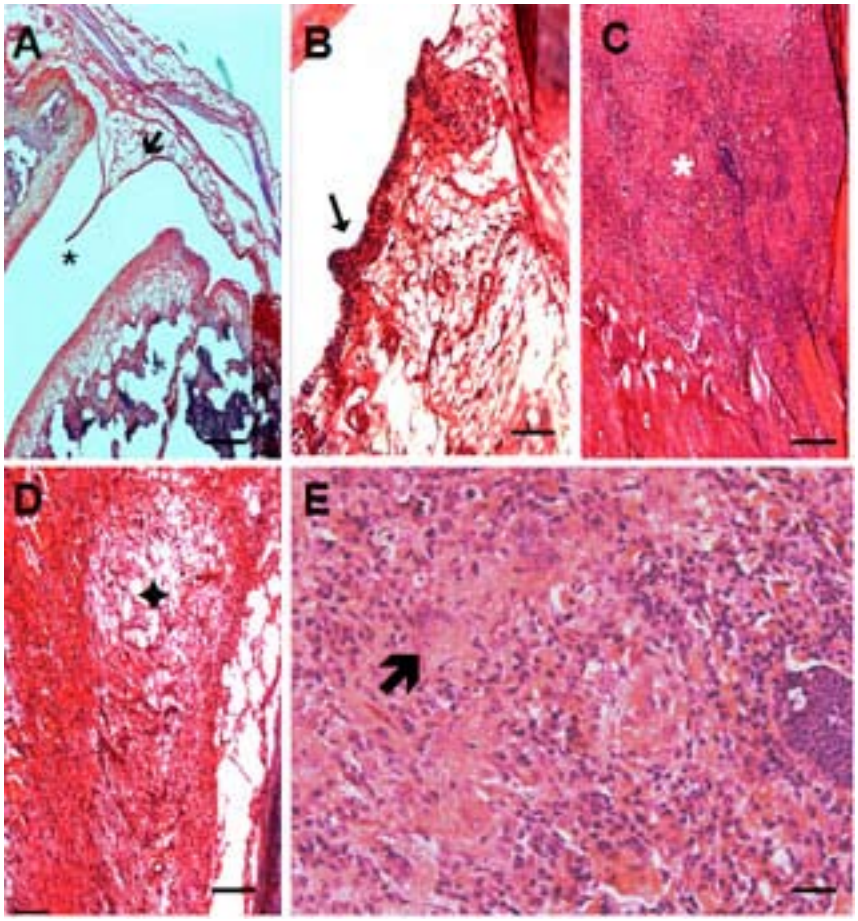

Figure .2 Histological section of knee joint change: (A) normal knee joint of an unijected rat showing condyles laterally (*) and subsynovial areolar tissue above (è). Hematoxylin and eosin, score bar 10 µm. (B) Zymosan –induced arthritis in a kenee join 3 days postinjection showing synovial lining hyperplasia (¨) and low score for cellular infiltration. Hematoxylin and eosin score bar 5µm. (C) Zymosan induced arthrits after 7 days postinjection showing subsynovial and periarticular tissue contained a moderate infiltration infiltrate histiocystes with patchy areas of PMN aggregation (Ù).(è). Hematoxylin and eosin score bar 5µm. (D) Zymosan-induced 14 days after injection in knee joint changes of synovial hypertrophy and pannus. The inflammation focus is organized with predominance of giant cells granuloma (è). Hematoxylin and eosin score bar 5µm. (E) Detail of granuloma in synovia (æ). Hematoxylin and eosin score bar 2,5µm

mental arthritis, it was necessary to choose a functional model, of easy reproducibility, fast induction and that would not be burdensome. Analyzing these premises [5, 11], the choice was for a model of induced arthritis that would use wisar rats, due to their easy acquisition and handling [2, 31]. In the search for an ideal inductive agent, after some pilots, it was confirmed that Zymosan produced the inflammation that was pertinent to the contents of this study. The developed biological alterations should allow the accompaniment both in the acute and the chronic phases [17]. The first evidences of the inflammatory signals were proved when 48 hours after intra-articular induction by Zymosan, there was loss of function, observed through the time of paw raise (TEP) that was maximum for 8 hours after the incident [25]. The inflammatory process continued its evolution after chronicity of the inflammatory process was observed on the 14th day [22]. Mastocytes are degraded [4, 16] and increase the cellular permeability [8], taking an experimental model to the inflammation after being submitted to intra-articular injections by Zymosan [12,3]. In 1994 it was proved that 2mg Zymosan intra-articular injection produces acute inflammation and periarticular intra-articular on day zero, followed by sub-acute erosive synovitis [13].

Using (30) rats submitted to experimental arthritis induced by collagen, proved the harmful effect of TNF - and IL-1α in the synovial tissue and the cartilage, reduced in intensity after use of IL-4, and suggested research aiming at the use of this cytokine as a potential chondroprotective therapy [12, 23, 24-32].

In the injury of the cartilage by Zymosan, there are hystopathological, hystochemical and biochemical characteristics that are applied to the experimental study of RA and that, partly, meet the minimum requirements considered by Oliver and Brahn (1996) for the study of the human disease in animal models. In this experimental study, the arthritis induced by Zymosan, uses, as parameter, the cellular migration, radiological images and the inflammatory signals. During the induction process, after inoculation with Zymosan, on the 3rd day we found: moderate inflammation, synovial and villous hyperplasia, unspecific chronic inflammatory process [17, 18], soft juxtaposed tissues or para-articular with unspecific inflammation with some rich PMN in neutrophils. As the synovium is approached, the amount of neutrophil diminishes [27]. In osseous cork l, the periosteum appears swollen. Predominance of broken up neutrophil [2, 14]. On the 7th day after inoculation, we noticed infiltrated inflammatory unspecific diffuse lymphoplasmocytosis with intense inflammation, beginning of inflammatory flake organization. Inflammation rich in neutrophil. Inflammatory process, soft tissue parts articular joust and synovium [26] Intra and peri-articular inflammation, presence of giant cells. Inflammation 2 and 3 degree. On the 14th day after inoculation, we found infiltrated inflammatory focal lymphoplasmocytosis forming aggregate. Adipose peri-articular tissues. Villous hyperplasia. Degree 3 intense inflammation localized in the synovial, muscular, peri-articular tissue. Inflammation focus organized with predominance of giant cells. Appearance of marginal erosions [31, 32] in the osseous tissue. Presence of pannus. Lymphocytic inflammation, productive granuloma, non- caseous, ample and rose cytoplasm of Langhans cells. Epithelioid cells. Presence of giant cell granuloma [8].

Cellular giant reaction with granuloma of strange body with intense necrosis area. articular destroyed surface exposing the reactive neoform osseous tissue [13, 14, 15, 17, 18, 26-3].

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